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Article

Evaluation of the fungal microflora infesting pigeon pea (*Cajanus cajan* L. Millspaugh) in southern Benin and associated mycological hazards

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Abstract

Pigeon pea is a perennial legume with a good nutritional value. Unfortunately, it is also a substrate for fungi contamination. Then, a qualitative semi-structured survey was carried out in the main production areas of pigeon pea in southern Benin. This survey was coupled with samples collection. A total of 60 samples of pigeon pea were collected and analyzed for associated fungal microflora by using a taxonomic schemes primarily based on morphological characters of mycelium and conidia. Obtained results indicated a low technological valorization of pigeon pea seeds in southern Benin and their used only in direct consumption after cooking. Microbiological analyses revealed the high contamination of pigeon pea seeds by fungi, with the most occurrence of Aspergillus (71.42%), followed by Fusarium (26.19%). Fungal species such as Aspergillus ochraceus, A. parasiticus, A. flavus and Fusarium oxysporum were also detected in analyzed samples. Taking into account the toxicity of the secondary metabolites produced by these fungi, mycological hazards are discussed and important methods for the control of mycotoxin-contamination are further provided. More attention should be paid to the mycological quality of this legume, in order to protect the consumers' health.

1 Introduction

The nutritional value of legumes has considerable worldwide interest, due to the demand for healthy food (Nestel et *al.,* 2004). Legumes are good sources of protein and dietary fiber with high levels of vitamins and minerals; and also contributed to the control of certain metabolic diseases (Almeida- Costa et al., 2006). Pigeon pea (*Cajanus cajan* L. Millspaugh) is a legume belong to the family of *Fabaceae* (Wu et *al*., 2009). It is often cultivated in tropics areas, including the semi-arid one. Its annual production, estimated at 3.1 million tons, accounts for about five percent of world seed legumes production. It has the fifth place among the legumes and contributes to thirty-three percent for the nitrogen requirements for human food (Fossou et *al.,* 2012).

In developing countries, interest in the culture of pigeon pea is justified by many opportunities it offers to rural populations (Fossou et *al.,* 2012). Indeed, it is one of the most attractive crops for African agriculture, due to its simple low-cost production adapted to sub-Saharan climates, its nutritional value, its exceptional capacity for soil regeneration and its diversified uses for men and cattle (Pazhamala et *al.,* 2015). It is grown in more than twenty five tropical and subtropical countries, either in monoculture or in rotation with cereals or other legumes.

Pigeon pea is mainly grown for its seeds, whose nutritional value is comparable to that of beans (*Phaseolus vulgaris*) (Fossou et *al.,* 2012). Indeed, known to be an excellent source of protein (21.7%), pigeon pea are also a good source of energy, vitamins and essential amino acids such as lysine, phenylalanine, valine, leucine and isoleucine (Wu et *al*., 2009). The seeds are rich in fatty acids, such as linoleic and palmitic acids; and are also a good source of iron and calcium (Fossou et *al.,* 2012).

Unfortunately, like other legumes often grown in Benin such as peanut and cowpea, pigeon pea is also a preferred substrate for parasites, such as fungi. This contamination could also be associated with mycotoxin production (Sultan and Magan, 2010). Mycotoxins are secondary metabolites of low molecular weight, which are present in many food and feed products and can cause many diseases for humans and animals (Wagacha and Muthomi, 2008). These molecules are not destroyed during prolonged storage and are often resistant to thermal or chemical treatments (Cahagnier *et al.,* 1998). The main aim of this study was to evaluate the fungal microflora infecting pigeon pea seeds at post-harvest in Benin, as well as associated mycological hazards. Findings will serve the purpose of alerting consumers on the dangers of consuming poorly stored grains.

2 Material and methods

2.1 Survey

A qualitative semi-structured survey was carried out in the main production areas of pigeon pea in southern Benin, including six locations (*Kétou, Pobè, Agouna, Klouekanmè, Azovê* and G*lazoué).* A total, eighty (80) stakeholders of the sector, including farmers (production

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sites), traders and consumers (markets) were surveyed. The survey was carried out through individual interview by using a pre-established survey form. The searching information concerned: different types of varieties encountered in the areas investigated, parasitic factors of pigeon pea, conservation methods and different uses of the pigeon pea seeds.

2.2 Collection of samples

A total of 60 samples (each 500 g) of pigeon pea were purchased from the different investigated localities. The samples from ten points were collected. Each sample was shelled in a sterile flow bench to obtain the pigeon pea grains which were kept at 4 °C until fungal enumeration.

2.3 Fungal isolation and identification

Direct plating technique described by Pitt et al. (1994) was used to examine samples. One hundred pigeon pea grains per sample were surface disinfected in 0.4 % active chlorine solution for 1 min at room temperature. Then, they were placed directly on Yeast Extract Sucrose Agar medium (YES). Plates were incubated at 25°C for 5 to 7 days. This method permits recovery of the fungi actually growing in the particles. The dilution plating method was also used in other to recovery of the fungi growing on the particles as described by Nguyen (2007). Fungi were purified by repeated subcultures. Pure cultures of fungi were examined macroscopically and microscopically and their identification was carried out by using a taxonomic schemes primarily based on morphological characters using the methods given by Singh *et al*. (1991), Filtenborg *et al.* (1995), and Tabuc (2007).

2.4 Statistical analysis

Experiments were performed in triplicate, and data analyzed are means subjected to one-way Anova. Means are separated by the Tukey's multiple range test when Anova was significant ($P<0.05$) (SPSS 10.0; Chicago, IL, USA).

3 Results

3.1 Results of survey

The results of the qualitative semi-structured survey carried out in the production areas of pigeon pea in southern Benin have revealed that there are mostly three types of pigeon pea varieties that are commonly encountered in investigated areas. These varieties are characterized by their color of seeds (whitish color, whitish color stained with black and brown color - Figure 1). However, the preference for each type of pigeon pea grain varies according to investigated zone. Indeed, for grains of whitish color, the preference, according to the persons

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surveyed, is 40% for Ketou, 40% for Pobè, 70 % for Agouna, 70 % for Azovè and 70% for Glazoue (Table 1). Moreover, in the zone of *Klouekanmè*, only the whitish color variety is mostly encountered. According to the questionary surveyed, this variety is much appreciated because its seeds have a faster cooking time with a more attractive taste. The surveys have also indicated that there is no other form of upgrading of pigeon pea in southern Benin. The only form of use this legume is the direct consumption after cooking. Besides pest's attacks by weevils (*Callosobruchus maculatus*), the survey also underlined the contamination of the seeds by fungi. This contamination is often characterized by the presence of black spot on the seeds, especially in post-harvest. The survey have also revealed that all farmers surveyed used chemical insecticides to control pest's damage of pigeon pea in postharvest.

Figure 1. Color varieties of pigeon pea commonly encountered in investigated areas

a- whitish color seeds   b- whitish color stained with black seeds   c- brown color seeds

Table 1: Preference of surveyed people in each investigated area for different type of pigeon pea seeds (%)

Localities	Whitish color seeds	Whitish color stained with black seeds	Brown color seeds
Ketou	40^a	10^b	50^c
Pobe	40^a	00^b	60^c
Agouna	70^a	23^b	07^c
Kloukanmè	100^a	00^b	00^b
Azove	70^a	30^b	00^c
Glazoue	70^a	20^b	10^b

Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests.

3.2 Results of identification of fungal microflora

The result of microbial analysis and isolation of fungi in pure culture indicated that pigeon pea samples collected from investigated areas were highly contaminated by fungi (Table 2).

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The most prevalently fungi recorded, with statistically significant difference, are *Aspergillus ochraceus* (23.80%), *Fusarium oxysporum* (19.04%) and *Aspergillus flavus* (16.66%) (Table 3). The most prevalently genera was *Aspergillus* (71.42%), followed by *Fusarium* (26.19%).

Table 2: Prevalence of isolated fungi taking into account the collection zones

Collection areas	Fungi isolated	Prevalence (%)
Agouna	Aspergillus ochraceus	33,33a
	Aspergillus tereus	16,66b
	Aspergillus niger	33,33a
	Fusarium oxysporum	16,66c
Glazoué	Aspergillus ochraceus	25a
	Aspergillus fumigatus	12,5b
	Aspergillus ustus	12,5b
	Fusarium oxysporum	37.5c
	Fusarium spp.	11,11b
Azovê	Aspergillus flavus	22,22a
	Aspergillus oryzae	11,11b
	Aspergillus candidus	11,11b
	Aspergillus ochraceus	22,22c
	Fusarium oxysporum	22,22c
	Fusarium verticilloides	11,11b
Klouekanmè	Aspergillus tereus	14,28a
	Aspergillus parasiticus	14,28a
	Aspergillus oryzae	14,28a
	Aspergillus ustus	14,28a
	Aspergillus ochraceus	28,57b
	Fusarium oxysporum	14,28a
Kétou	Aspergillus niger	16,66a
	Aspergillus flavus	33,33b
	Aspergillus ochraceus	16,66a
	Fusarium oxysporum	16,66b
	Mucor spp.	16,66a
Pobè	Aspergillus flavus	66,66a
	Aspergillus spp.	16,66b
	Aspergillus ochraceus	16,66b
	Fusarium verticilloides	16,66b

Values are means. The means followed by same letter in the same group (collection zone) are not significantly different according to ANOVA and Tukey's multiple comparison tests.

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Table 3: Prevalence of isolated fungi from collected pigeon pea

Fungi isolated	NCA	Prevalence (%)
Aspergillus ochraceus	10	23.80a
Aspergillus flavus	7	16.66b
Aspergillus niger	3	7.14c
Aspergillus oryzae	2	4.76d
Aspergillus terreux	2	4.76d
Aspergillus ustus	2	4.76d
Aspergillus parasiticus	1	2.38e
Aspergillus fumigatus	1	2.38e
Aspergillus candidus	1	2.38e
Aspergillus spp.	1	2.38e
Fusarium oxysporum	8	19.04b
Fusarium verticilloides	2	4.76d
Fusarium spp.	1	2.38e
Mucor spp.	1	2.38e

NCI : Number of cases of isolation out of 60 samples. Values are means. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

4 Discussion

Increasing interest in finding new food sources to alternative malnutrition in developing countries has observed in recent time. According to Naylor et *al*. (2004), some of Africa's native drought-tolerant crops are also some of the least researched worldwide and are thus referred to as "orphan crops". This study, which focus on the valorization of the neglected and under-utilized species of Benin such as pigeon pea, has indicated that different varieties of this legume, often considered as a secondary crops, are not fully known by the Beninese populations. It culture has not also been extensively focused by scientific researches as it is the case in some countries such as Nigeria (Ade-Omowaye et al., 2015) and Uganda (Velay et al., 2001). However, cultivation of pigeon pea has been also reported in Niger, Mali (Versteeg and Koudokpon, 1993), Ethiopia, Zimbabwe (Kamanga and Shamudzarira, 2001), Zambia (Boehringer and Caldwell, 1989), Botswana (Amarteifio *et al*., 2002), and South Africa (Swart, 2000). The results from the present study also indicated the low technological valorization of this food resource, which is used exclusively for direct consumption after cooking. Therefore, these findings underlined the need to use scientific knowledge and biotechnological tools to value this important food resource.

However, like other legumes, the results from this study also indicated the infestation of pigeon pea by insects as well as fungi. These findings are also in accordance of those reported by Hubert et al. (2007) which underlined the implication of weevils in the contamination of

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crops by fungi. The results from the isolation and identification of fungi in pure culture have indicated the contamination of pigeon pea collected in southern Benin by fungi, with a high occurrence of *Aspergillus* strains (71.42%). This contamination is characterized by the presence of *Aspergillus parasiticus*, *A. flavus* and *A. ochraceus* (Table 3). This high contamination by *Aspergilla* strains could be in relation with the type of fungi presents in the soil of culture areas. The toxinogenic potential of these fungi have been reported by Adjou *et al*. (2012) in peanut cakes and also in peanut seeds collected from Benin (Adjou *et al*., 2013). These fungi can produce a significant amount of mycotoxins, when food storage conditions are inadequate.

Review of scientific literature on mycotoxin related human diseases, clearly reveals a linkage between ingesting of mycotoxin-contaminated food and illness, especially hepatic, gastro-intestinal, carcinogenic and teratogenic diseases. Among these mycotoxins, Aflatoxins (AFs) produced predominantly by *Aspergillus parasiticus* and *A. flavus* are highly toxic as they are carcinogenic, teratogenic, and causing human liver and extra-hepatic cancers (Castegnaro, 1999). Other mycotoxins such as Ochratoxin A (OTA) and citrinin (CIT) produced by *Aspergillus ochraceus*, *A. carbonarius* and *A. niger* (Pfohl-Leszkowicz, 2002) are also detected in food. The co-contamination of fungi strains which can produce aflatoxins and ochratoxin A in pigeon pea seeds should be taken into consideration. This is particularly important in regard to possible synergism and additive effects of these mycotoxins. Such co-contamination has been previously observed with other food samples, such as breakfast cereal (Molinie et al., 2005), olives (El-Adlouni et al., 2006) and peanut cakes (Adjou et al., 2012). This makes it important to avoid the conditions that lead to mycotoxin formation at all levels of production, harvesting, transport and storage, which is not always possible and not always achieved in practice. According to Fernández-Cruz *et al.,* (2010), environmental stress conditions such as insect infestation, drought, cultivar susceptibility, mechanical damage, nutritional deficiencies, and unseasonable temperature, rainfall or humidity can promote mycotoxin production in growing crops. In fact, changes in farming practices in the past few decades may result in increasing stress on plants and therefore enhance fungal invasion and mycotoxin contamination (Fernández-Cruz et al., 2010).

5 Conclusion

This survey underlined the low valorization of pigeon pea in southern Benin and its contamination by fungi, which could be resulted in mycotoxin production. The control of mycotoxin-contamination in agricultural products must taking into account the good agricultural practices which include early harvesting, proper drying, basic sanitation measures (removal and destruction of debris from previous harvest) and proper storage (adequate drying, elimination of insect activity). The biological control through the development of atoxigenic bio-control fungi that can out-compete their closely related, toxigenic strains in field environments, could also be used.

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